THE SPORANGIA OF THISMIA AMERICANA

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(WITH PLATE XVI)

Of the investigations among Burmanniaceae, the morphological studies of TREUB, JOHOW, and ERNST and BERNARD are prominent. These studies included both chlorophyllous and dependent forms, although the latter are better represented. The accounts vary considerably in completeness, since in the earlier ones close stages are sometimes lacking.

That there is variety within the family in development up to the mature seed is evidenced in the widely different accounts for those forms in which there is no evidence of fertilization, as compared with those where this process undoubtedly occurs. The net product seems to be approximately the same, that is, a small mass of endosperm cells about an embryo of from 2–10 or more cells, usually with no differentiation. A striking exception occurs in *Thismia clandestina*, which has a 3-celled suspensor and a spherical body differentiated into 2 layers. As in Orchidaceae (12), however, the preliminaries to this vary. Division of the megaspore mother cell may produce a row of 2 cells (as *Burmannia candida*, 5), in which the inner cell gives rise to the embryo sac; or a row of 3 cells, the innermost of which, a true megaspore, functions in producing the female gametophyte; or the usual tetrad of angiosperms, of which the innermost megaspore is functional.

In the production of these cells the mother cell may go through a reduction division (as *Burmannia Championii*, 5), in which case fertilization is the rule; or it may divide by an ordinary mitotic division, so that the progeny have the double number of chromosomes rather than the reduced number (*Burmannia coelestis*, 2).

In all cases the embryo sac mother cell, whether a megaspore or the result of a single division of the archesporial cell, develops by 3 consecutive divisions to produce the 8-celled stage. Polarity is early evident, and the egg apparatus is organized, with small

antipodal cells at the opposite end of the sac, while the 2 polar nuclei usually meet near the center, sometimes nearer the chalazal or micropylar end (as *Burmannia Championii*, 5).

When the egg is mature, in some cases there is evidence of the entrance of a pollen tube with the discharge of two male cells, one of which fuses with the egg, the other with the polar nuclei, as *B. candida*. That the latter fusion is not a complete one is held by ERNST and BERNARD, who see in a 3-parted nucleus with 3 nucleoli evidence against entire merging, at least in the first divisions of this endosperm nucleus. The fusion of the egg nucleus, however, is slower here than that of the 3 nuclei in the center of the sac. When there are 2–4 cells in the endosperm, the sex nuclei still remain distinct in *B. candida* (5). Later the fertilized egg gives rise to an embryo of 2 or more cells, varying with the form studied.

In cases where no fertilization has been observed there was development of seeds as indicated, except that no fusion save that of the polar nuclei occurred. *Thismia javanica* (3) and *Burmannia coelestis* (2), examples in which this condition holds, show no reduction division in the formation of the "megaspore." This condition is the one to be expected from such work as has been done in parthenogenetic angiosperms. The development of the seed is first evidenced in *B. coelestis* by the division of the endosperm nucleus, which usually results from the fusion of 2 polar nuclei; occasionally there are more than the two concerned, as 3–5, probably through the functioning of synergids or antipodal cells. Thereafter the development seems much as in sacs where fertilization has taken place. ERNST and BERNARD in their series of studies of Burmanniaceae report for *B. coelestis*, *B. candida*, *B. Championii*, *Thismia clandestina*, *T. Versteegii*, and *T. javanica*, practically the same sort of development in the endosperm region, regardless of the introduction of a male cell. The first division of the fusion nucleus gives rise to 2 nuclei, the lower of which is cut off by a wall. The cell thus formed is designated as the "basal apparat" or haustorium cell. The other nucleus, however, continues to go through successive divisions in which no cell plate is formed, with the result that there are a number of free nuclei in the endosperm region. Walls then develop in this region at approximately the same time or a

little before the beginning of nuclear division in the embryo cell proper. The extent of tissue development in *B. Championii* may be judged by ERNST and BERNARD'S statement that there are 6–8 cells in the median longitudinal line in the mature sac, and that *B. coelestis* has about 30 endosperm cells at maturity.

The antipodal cells, never conspicuous, usually appear in a little V-shaped region below the haustorium region, sometimes as a row of cells, more frequently as two cells above one.

The cell giving rise to the embryo, whether after fertilization or not, goes through at least one nuclear division, and usually more. *Gonyanthes candida*, as reported by TREUB (13), develops a 2-celled embryo; as reported by JOHOW, and again by ERNST and BERNARD (as *B. candida*), it has a 3-celled embryo. JOHOW (8) found in *Gymnosiphon tenellus* a 3-celled situation similar to B. *candida*, and in *Dictyostegia orobanchioides* and *Apteria setacea* a 4-celled embryo, comparable to that found in *B. javanica* by TREUB (13). *Gymnosiphon trinitatis* (8) and *Thismia javanica* (3) show slightly greater development in a 6 or more-celled embryo, whereas *Thismia clandestina* (4) shows the greatest differentiation in a structure consisting of a 3-celled suspensor and a spherical body in which a single outermost layer of cells is differentiated from the inner mass. There is a striking similarity to Orchidaceae (12) so far as extent of development of the embryo is concerned. The contrast in the mature seed, on the other hand, due to failure of endosperm development in Orchidaceae, is equally noticeable. JOHOW, and later ERNST and BERNARD, have described the development of a small "nucellus polster" above the embryo sac, and an even more conspicuous tissue at the chalazal end. The possibility of the functioning of the latter at the time of germination of the seed as a region of water transfer (the rest of the tissue shows great cutinization) has been suggested, although no evidence of experimental character has been forthcoming. In contrast to the striking nucellus tissue at the ends, there is very evident degeneration of the cells in the middle zone or ring, as in *Gymnosiphon*, *Burmannia candida*, and *Thismia clandestina*.

In comparison with the thorough work done on embryo sacs, the scant attention paid to the pollen situation brings forth prac-

tically only the method of pollination and fertilization where this process occurs. This has been reported by several workers: MIERS (9) in *Dictyostegia orobanchioides*, WARMING on Brazilian forms and in *Apteria lilacina*, and ERNST and BERNARD in *Burmannia candida* and *B. Championii*. In all these forms germination of pollen occurs in the pollen sacs, so that the tufts or bundles of pollen tubes issue from the anthers and penetrate the stigma. MIERS remarked that the identity of these pollen tubes is clear with the use of a common lens, while the cottony mass of threads is evident, supposedly to the naked eye. He distinctly stated that this is not true, however, in *Myostoma* and *Ophiomeris* (9), and took this as evidence, in his early time, that thereby "the theory of the application of pollen tubes for the fertilization of its ovules is distinctly disproved." ERNST and BERNARD were unable to discover this method of pollination in *Thismia javanica* or *T. clandestina*, although aware of its presence in other forms and so alert for indications here. So far the evidence goes to show that such early germination of pollen and subsequent growth occur only in Euburmanniae, where the structure of the flower is different from that in *Thismia*. There seems to be a general conclusion, however, that forms are self-pollinated, through evidence such as given by SCHLECHTER in *Thismia Winkleri* (1, 11), where little diptera were found in the base of the flower where the pollen must fall.

Investigation

The material upon which the present study is based is that of *Thismia americana*, collected by the writer in Chicago, Illinois, during the summers of 1913 and 1914. The relationships of this form and a description of its structure, etc., were given in a previous paper (10).

In very young stages the stamen set appears to be distinct earlier than the ovary parts. Each stamen, of which there are 6, produces the usual 4 microsporangia, all of which are directed away from the central axis of the flower. Thus the surface of the anther toward the center is quite flat or slightly concave, while the opposite one is marked by the 4 lobes, in 2 pairs, which represent the rudiments of the microsporangia. At this stage usually the

connectives have not become so broadened as later, so that the individual stamens appear more distinct than the tube shows at maturity. The youngest stage where differentiation appears is indicated in fig. 14, where hypodermal masses of meristematic cells, separated from each other by a double layer of sterile cells, appear beneath a distinct, large-celled epidermal layer. At this time the ovary chamber is just beginning to show distinctly with the 3 placentae, which later give rise to the ovules projecting inward. Later the individual sporangia show the parietal layer to be but 2 layers thick, within which there is a conspicuous tapetum, while outside of it is the epidermal layer (fig. 15). The tapetum shows dark irregular bodies which may represent waste or reserve material. At this stage it is evident in many preparations that not all of the tissue originally differentiated as "sporogenous" is fertile. A number of the spores abort, so that in any one section only a few appear normal (fig. 16). Often between adjacent cells small oil globules appear as extraneous matter, possibly released through changes due to degeneration of the spores.

The microspores are shed from the stamens through a longitudinal dehiscence of the anther. At the time of shedding one division of the microspore nucleus has taken place in such as appear functional. The tube and generative nuclei can be distinguished quite readily, although often other bits of dark staining material are present.

Germination of pollen grains with formation of fine pollen tubes has been observed. By dissection of the style several tubes were traced through to the ovary cavity. At this time practically all the pollen had been shed from the stamens of the flowers under consideration. It seems likely that there is self-pollination as in other forms. The contrast with the Euburmannia forms reported lies in failure of development of the mass of pollen tubes from the microsporangia to the stigma, as reported by MIERS and ERNST and BERNARD. The structure would practically bar such a possibility, since the greatly developed stamen tube arising from the connectives usually extends below the level of the stigma. The dehiscence of the microsporangia occurs on the face away from the central region in which the style is erected, and the pollen falling from the

sacs would naturally drop to the floor of the cavity, that is, the roof of the ovary. In this fall it is obvious that the grains cannot come in contact with the stigma, which is separated by the stamen tube, although grains have been observed along the style. The cells of the inner surface of the stamen tube are often glandular in nature (fig. 16), although this would seem to have no special significance except in connection with the entrance of insects. It seems likely that the latter are necessary agents in pollination because of the mechanics involved.

The placentae which appear in the ovary during the development of the microsporangia give rise after a time to the primordia of the ovules (fig. 1). The surface of the placentae first becomes uneven through the appearance of the little lobes marking the rudiments. Soon the inner integument appears, and finally, as the ovule assumes the anatropous orientation, the outer integument is quite distinct except on the side where the funiculus appears. Meanwhile the hypodermal archesporial cell has become differentiated (fig. 2). The condition of mother cells usually occurs in the stamens at the same time that this archesporium appears in the ovule (cf. figs. 2 and 15). This cell represents the megaspore mother cell directly, since no parietal cells are developed here. It enlarges noticeably, and at length undergoes nuclear division, during which the chromatic material becomes massed at one side of the nucleus in synapsis (fig. 2). After division two cells separated by a thin wall are evident (fig. 3). At the same time the whole ovule is developing rapidly, as shown by the spindles in the tissue about the megaspore mother cell or its progeny. The two daughter cells divide further. The spindle in the outermost cell is oriented at right angles to the long axis of the ovule, that of the inner parallel to this axis. The result is that there is a pair of megaspores side by side which frequently are so crushed together in later stages that they lie obliquely (fig. 5) or appear finally as one (fig. 10). Sooner or later these cells disorganize, as does the sister cell of the functional megaspore, which lies innermost in the series of four. The pressure of development usually shows first on the outermost megaspores (fig. 5), but sometimes the third non-functional one is crushed first (fig. 6).

At the time of the first division of the megaspore developing the gametophyte, the abortive cells are dark staining, often wholly disintegrated masses of material. The binucleate stage shows nothing unusual, with its tendency toward polarity with the appearance of a central vacuole (figs. 8, 9). This stage is followed by the usual 4-nucleate situation arising from the division of each of the nuclei (fig. 10). The 4-nucleate phase must give rise very soon after formation to the 8-nucleate, since it represents a difficult stage to find.

The early 8-nucleate stages (fig. 11) show 4 free nuclei at each pole, with a large central vacuole. This is followed by great enlargement and the organization into an embryo sac of the typical form of angiosperms, the egg apparatus at the micropylar end consisting of 2 large synergids in contact with the egg, 3 smaller free antipodal nuclei in the narrower, more pointed chalazal end of the sac, and 2 polar nuclei, usually coming in contact with each other near the micropylar rather than the chalazal end (12). Stages both before and after the fusion of these polar nuclei have been found. The peculiar lobed effect reported by ERNST and BERNARD in *Burmannia candida* and interpreted there as incomplete fusion is sometimes evident here. That there is any special significance here seems doubtful.

At this time the cells surrounding the embryo sac stain more deeply and stand out more sharply than in younger stages. So far fertilization stages have not been observed. Contrary to ERNST'S report of development in *Burmannia coelestis*, it seems altogether likely that fertilization does occur, since pollen tubes are developed.

The development of the seed has not been followed in detail. At one time the larger portion of the sac is filled with the free nuclei resulting from the division of the endosperm nucleus. Soon walls come in, forming large cells. At about this time the egg cell undergoes division, so that a 2-celled proembryo is present imbedded in the conspicuous endosperm tissue. Further division occurs in the proembryo cells, and in the oldest material obtained (presumably mature seeds, although not so proved by germination) the embryo consists of many cells in a globular mass with a short suspensor region (fig. 13). The situation is much like that in

Thismia clandestina. The endosperm is packed with reserve material at this time, and stains very deeply as a result.

The development of the nucellus and integument into peculiar layers has been noted under the literature of other forms. In *Thismia americana* there is also at maturity a distinct mass of irregular small cells at the base connecting by means of a dark staining nucellar layer with a cap of peculiar cells at the micropylar end. The nucellar layer next to the endosperm shows fungal hyphae and many oil bodies as part of the contents. Gelatinization of the walls at the chalazal end begins early, and is responsible to some extent for the prominence of the mass of cells at that end.

Enough material has not been available to try a satisfactorily large range of germination experiments. Those which have been tested have given negative results. In all probability, as in orchids, the fungus plays a rôle in the early development of the plant.

Summary

1. In the microsporangia the sporogenous cells develop from hypodermal masses, 4 in number, in the usual fashion.

2. At maturity the innermost parietal layer appears crushed by the large tapetal cells.

3. There is marked abortion of sporogenous cells in the microsporangia.

4. The division of the megaspore mother cells gives rise to 4 megaspores, the outer 2 oriented at right angles to the long axis of the ovule.

5. The 3 outer megaspores degenerate very soon, disappearing entirely after a short time.

6. The functional megaspore divides in the usual way, so that eventually an embryo sac of 8 nuclei is produced.

7. Presence of pollen tubes makes fertilization seem likely.

8. The well developed embryo is imbedded in large endosperm cells which are conspicuous in storage contents.

9. In the seed the nucellus makes a conspicuous layer, developing into a cap of tissue at each end.

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EXPLANATION OF PLATE XVI

All figures were drawn with the aid of the camera lucida, and show magnifications as follows: figs. 1, 3, 5, 10, ×840; 13, 14, 15, 16, ×500; 17, ×260; 2, 7, 8, 9, 11, ×916; 4, 6, ×784; 12, ×1651.

FIG. 1.—Primordium of ovule.

FIG. 2.—Synapsis in megaspore mother cell.

FIG. 3.—Daughter cells of megaspore mother cell.

FIG. 4.—Four megaspores.

FIG. 5.—Four megaspores, two outer cells already disorganized.

FIG. 6.—Same stage, but sister cell to functional megaspore crushed.

FIGS. 7–9.—Binucleate embryo sacs.

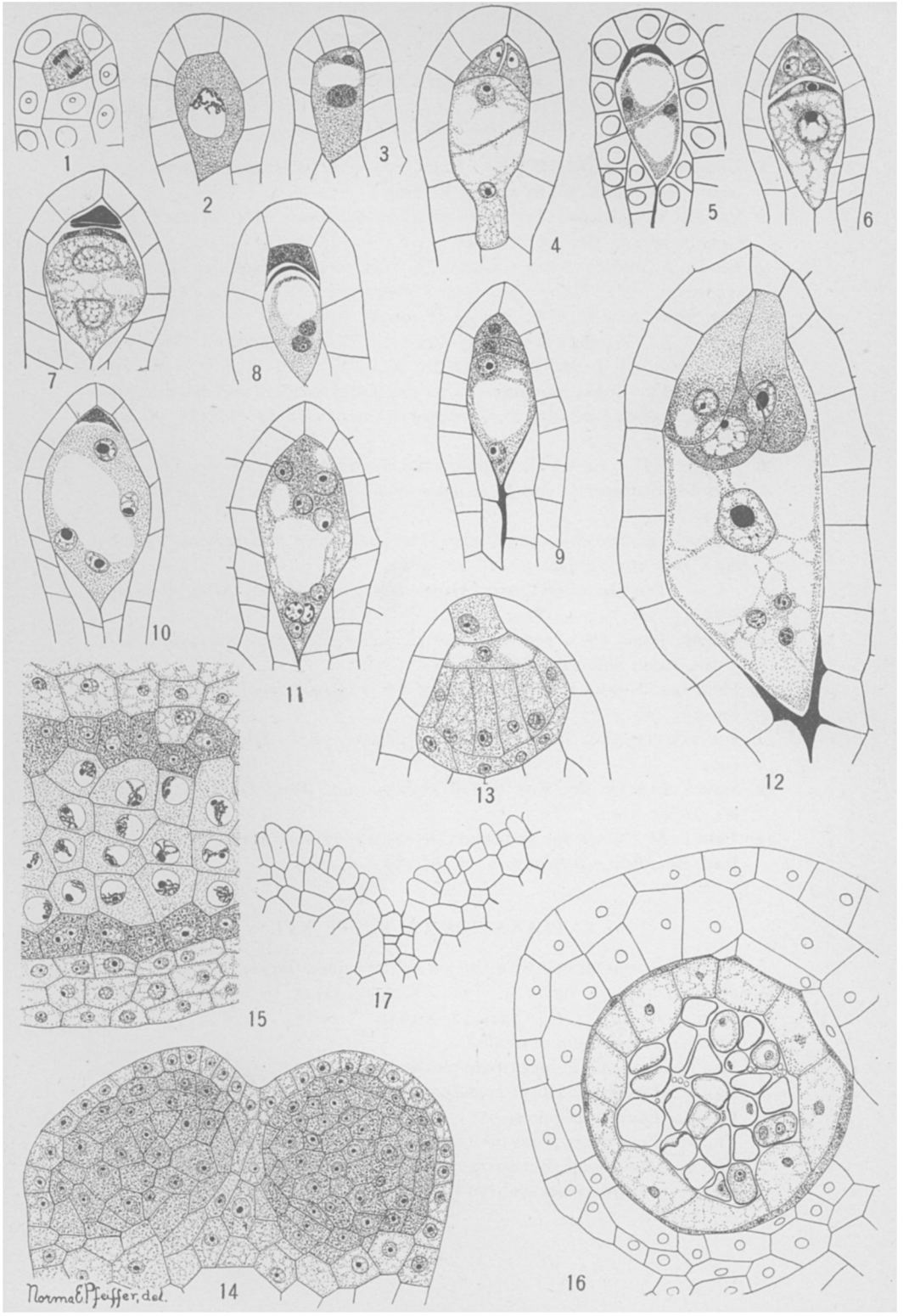

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FIG. 10.—Four-nucleate embryo sac; non-functional megaspores disorganized.

FIG. 11.—Eight-nucleate embryo sac.

FIG. 12.—Embryo sac at maturity; chalazal walls conspicuously gelatinized.

FIG. 13.—Embryo.

FIG. 14.—Young anther, showing 2 of 4 microsporangia.

FIG. 15.—Microsporangium with mother cells in synapsis, longitudinal section.

FIG. 16.—Microsporangium, showing large number of sterile pollen grains, tapetum disorganizing.

FIG. 17.—Portion of stamen tube, showing glandular cells of inner surface (nearest style).